

The University of Chicago

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PITUITARY IN THE OPOSSUM AND ITS
RESPONSES TO HORMONAL
TREATMENTS

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN
CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

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NORMAL DEVELOPMENT OF THE PITUITARY IN THE OPOSSUM AND ITS RESPONSES TO HORMONAL TREATMENTS¹

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TWO PLATES (SIXTEEN FIGURES)

I. INTRODUCTION

The work of Smith ('27, '30), Smith and Engle ('27), and Zondek and Ascheim ('27) may well be said to have initiated the intensified studies on the anterior pituitary during the last decade. Materials for these studies have been contributed by all classes of vertebrates, and have demonstrated many variations in gross and microscopic structure.

For the past few years a program of research has been carried out in this department which has utilized the American opossum (*Didelphys virginiana*). These studies, largely on the reproductive system, especially during the early developmental stages (Moore, '39; Moore and Bodian, '40; Moore, '41 a, b; and Moore and Morgan, '42) have made available the normal pituitary gland of this marsupial at various stages in ontogeny as well as glands subsequent to a variety of experimental treatments. Since the pituitary of the opossum has received relatively little attention (Dawson, '38) it appeared desirable to investigate several phases of it, if only in an introductory manner, in order to learn whether it presented especially favorable material for more intensive study at a later period. The earlier work of Parker ('17) provides considerable data of importance on the embryology of the pituitary gland of other marsupials.

¹ This paper is a condensation of a doctor's thesis of the same title, on file in the University of Chicago Libraries, Chicago, Illinois. The investigation has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

In this somewhat preliminary survey of the opossum pituitary attention has been given to such aspects as: (1) gross morphology and cytology of normal adults of both sexes; (2) effects of adult castration; (3) effects of hormonal treatments of adult females; (4) normal embryology and cellular differentiation; (5) the effects of hormonal treatments during the developmental period; (6) the effects of gonadectomy during early sexual differentiation.

It is an added advantage to have a knowledge of the reproductive systems of the animals whose pituitary glands are being studied because of the interrelationships which have been demonstrated between this gland and reproductive activity. Furthermore, cytological studies are useless unless correlated with functional studies.

This problem was suggested to me by Prof. Carl R. Moore. His constant cooperation in offering advice and criticism has greatly aided the progress of the study. I also appreciate the kindness of Prof. R. R. Bensley and Dr. Sylvia Bensley for their suggestions as to the possible identity of certain cytoplasmic inclusions. To Mr. Kenji Toda I am grateful for help in preparing the illustrations.

II. MATERIALS AND METHODS

The animals used in this study were kept under laboratory conditions and were obtained from various sources as described in previous papers (Moore and Bodian, '40; Moore, '41 a). The 100 pituitary glands thus far studied include normal adults: males—6, females—5; gonadectomized adults: males—2, females—6; hormone treated adults: females—14; normal immature animals (1–150 days): males—11, females—17, undifferentiated (1–11 days)—5; gonadectomized young: males—10, females—5; hormone treated young: males—10, females—9. In a few instances both a male and a female of the same age and treatment are compared, but in general only one animal in each category was available. For reports concerning the method of hormone treatment and castration see Moore ('41 a, b).

A. Histological procedures

The pituitaries from the earlier stages of pouch young were fixed by submerging the entire animal in Bouin's picro-acetic-formol. All of the glands taken subsequent to the fiftieth day were removed at autopsy and fixed in Zenker-formol. The tissues were washed, dehydrated with ethyl alcohol, cleared in oil of wintergreen, infiltrated with Parawax, and finally embedded in 58–62°C. paraffin. Heidenhain's modification of Mallory's connective tissue stain was employed for all tissues. Serial sections were cut at 4 and 5 μ . Whenever possible a few sections at 3 μ were cut at different levels of the gland. Nearly all sections were cut in a plane transverse to the long axis of the head, which is also transverse to the longest dimension of the gland.

B. The method of making cell counts

Three sections were selected for counting at representative levels in the gland. In cases where oblique sections were cut it was necessary to count a few more sections in order to correct for this error. A checker-board system of sampling was employed. Every other microscopic field in every other row of fields was counted. A binocular microscope with 6 $\times$ oculars and 1.8 mm. objective and a mechanical stage was used.

III. THE ADULT OPOSSUM PITUITARY

A. Morphology

First described by Dawson ('38), the opossum pituitary contains all of the usual components: anterior lobe, neural lobe, intermediate lobe, and pars tuberalis. The infundibular stalk is slender and contains a narrow infundibular recess. The solid infundibular process tips forward and is almost completely buried in the substance of the anterior lobe. A thin and very extensive intermediate lobe completely invests the neural lobe except for a small dorso-posterior portion. The residual lumen is wide and completely separates the intermediate lobe from the anterior lobe. The greatest length of the gland is parallel to the long axis of the body. Average

measurements of adult glands show the length to range between 4 and 5 mm., and the greatest diameter between 2.5 and 3 mm. The general shape of the gland is that of an oblate spheroid. The gland lies deeply buried in the basi-sphenoid bone.

B. Histology and the cell types

This study of the normal adult opossum pituitary is based on eleven animals, six males and five females sacrificed on the following dates:

MALES	FEMALES
(1) 1-10-41	(1) 6-10-40
(2) 4-30-41	(2) 5-2-41
(3) 4-30-41	(3) 12-15-41
(4) 6-21-41	(4) 6-5-42
(5) 6-21-41	(5) 6-7-42
(6) 7-1-41	

The anterior lobe consists of loose nests of chromophobic and chromophilic cells supported by a network of reticular connective tissue. There are numerous blood sinuses in the connective tissue. A differential distribution of the cell types exists, so that the extreme anterior end is largely basophilic, and the posterior end is decidedly acidophilic. Chromophobes are much more numerous in the basophilic end of the gland. Dawson also reported this distribution and local restriction of the cell types in the opossum anterior lobe. Although this restriction does exist, it is not complete, for almost any low power field in any part of the gland will reveal a few of all of the cell types.

The relative numbers of the various cell types are discussed in other parts of the paper, but it should be mentioned here that in normal animals in the breeding season the chromophiles are the dominant cells of the anterior lobe, whereas in the non-breeding female the chromophobes are the chief cells of the gland.

a. Acidophiles. The acidophiles contain a varying number of red-staining granules, from a very few to a climactic condition in which they are so closely packed that it is impossible

to distinguish individual granules. The nucleus is spherical and usually contains a central nucleolus and a varying number of large chromatin particles at the periphery and finer ones scattered throughout the nucleus. Some acidophiles contain dense chromatic nuclei which stain either blue or red. Exceptionally, the nucleus of an acidophile will appear as a colloid-filled ring. Acidophiles are also present which appear to contain colloid-filled vacuoles. For convenience in counting acidophiles were arbitrarily divided into two types, dark and light. The dark acidophiles stain heavily and individual granules are not readily seen. Light acidophiles are lightly stained and sparsely granulated so that each granule appears as a distinct dot. In normal glands the dark acidophiles are more numerous. As to size, acidophiles are intermediate between the small chromophobes and basophiles. They range in size from 8 to 11 micra with a nuclear size of 7 to 8 micra.

b. Basophiles. The blue-staining cells are the chief cells of the adult male and the breeding female; they have been divided into three principle types. In general, with the Mallory Azan technique, basophilic granules appear very rarely in the cells which stain with aniline blue. Cell counts reveal an average percentage of 0.50% of granular basophiles in the pituitaries of normal adults, while many glands have no granular basophiles in them. Granular basophiles are usually small, and the granules vary from light blue to dark purple; the nucleus is large, spherical, and does not contain a nucleolus. The majority of the basophiles of the opossum pituitary appears to have a fine-grained cytoplasm which stains blue but does not contain granules; these cells have been called the non-granular basophiles. The nucleus stains gray to pink, is spherical, and contains a central nucleolus surrounded by large chromatin particles. Negative Golgi are especially prominent and large in the non-granular basophiles. The third type of basophiles present in all normal glands is the colloid-containing type. These cells are as large as or larger than the other basophiles and contain droplets or a large pool of blue colloid, which may or may not be pierced

by clear, white vacuoles. At times, these cells may be extremely swollen to two to three times their normal size, and trabeculae of cytoplasm may be seen crossing the colloid from the patch of cytoplasm surrounding the nucleus to the cell membrane. In general, the nucleus is not of the pyknotic type, in which respect the cells differ from the so-called castration or signet ring cell. The colloid stains differentially light to dark which may be an indication of its physical state, that is, its degree of dispersion. Basophiles in the normal adult gland vary in size from 10 to 17 micra, while the nucleus is usually between 8 and 9 micra in diameter.

c. Chromophobes. These are the most inconspicuous cells of the anterior lobe. They appear as nuclei surrounded by very little cytoplasm. Chromophobes are rarely seen in nests in the opossum pituitary and almost always appear as interstitial cells packed in between the chromophiles. It is not unusual to see in the normal breeding male or female very large cells completely void of granules. These are not typical chromophobes, but are probably degranulated chromophiles (Severinghaus, '39). The typical chromophobes are the smallest cells in the anterior lobe, averaging 6 to 7 micra with a nuclear diameter of 5 to 6 micra; the large, degranulate cells are of basophilic proportions.

It should be emphasized that this classification of the chromophiles into different groups is an arbitrary one, and that it is supposed that they are not different cell types but rather cells in different phases of the secretory cycle.

Peculiar and consistent cytoplasmic inclusions appear in most of the chromophiles of the adult anterior lobe, which stain most often with aniline blue, at other times with azo-carmines or with Orange G. They appear as deeply staining, round masses, usually imperfectly round, but never with serrated edges. Prof. R. R. Bensley, and Dr. S. H. Bensley examined these inclusions and suggested that perhaps they were colloid gelations of varying consistency. The reasons for their difference in stainability might be due to differences in degree of dispersion, and therefore, the dyes with the

greater penetrating power would be able to stain them. These are merely suggestions and more study is indicated in order that their identity and possible significance be determined.

d. Epithelial cysts. Colloid-filled cysts are quite common in the anterior lobe of the opossum pituitary. More than one cyst may be found in the same gland. Cilia almost invariably occur in some of the cells lining the cyst. The general picture of the cyst wall is very similar to that of the naso-pharyngeal mucosa, with the exception that no goblet cells are present. Non-granular and colloid-filled basophiles are seen between the other cells lining the cysts in most instances. Similar cysts have been reported for the opossum by Martins ('33).

C. Effects of gonadectomy

Eight adults, two males and six females, were gonadectomized and left for periods varying from 30 to 136 days, to determine whether the changes so characteristic of other mammals occur in the opossum pituitary. Histological examination of the glands of these animals show definite changes of the usual type following gonadectomy (fig. 13). Adult opossums which are castrate for short periods of time do not show as great changes as those which have been castrate for longer periods of time. No differences in the response of male and female pituitaries following gonadectomy were observed. The females which were ovariectomized in the middle of the anestrus season and sacrificed 30 days later show an increase of basophiles from 19.92% to 55.50%, a very decided increase. It is significant that the greatest percentage of increase in basophiles is due almost entirely to an increase in the number of colloid-filled basophiles. In the early periods of castration the pituitaries do not exhibit the typical castration cells with pyknotic nuclei, although an increase in size and number of basophiles occurs. Following a period of 60 days of castration a decided decrease in the number of dark acidophiles occurs, and as in the longer term castrates, all of the cells take a lighter stain. The two females sacrificed 97 days and 136 days after ovariectomy show extreme differences from the normal;

pools of colloid within the basophiles have increased tremendously by this time, and vast numbers of typical castration cells with pyknotic nuclei have appeared. The acidophiles are obviously less prominent than in the normal gland, and sections show clumps of them invaded by swollen basophiles which appear to have borders of acidophilic granules. It is distinct that these are merely basophiles which have encroached upon acidophiles and have crowded them out of position. The Golgi apparatus in negative image becomes more distinct and increases in size following gonadectomy, as it does in pituitary cells of other mammals following gonadectomy.

The data collected by means of cell counts show unquestionably that a pronounced basophilia occurs in the adult opossum pituitary, both male and female, following gonadectomy of periods of from 30 to 136 days.

IV. NORMAL DEVELOPMENT AND CELLULAR DIFFERENTIATION

The early stages of the development of the hypophysis of marsupials was worked out thoroughly by Parker ('17).

This study of the normal development of the opossum pituitary is based on a series of thirty-three immature animals, ten males, eighteen females, and five undifferentiated. The description of the stages begins with the 1-day-old animals.

1st day. The pars tuberalis is tubular and extensive, and is still divided into two lateral extensions (fig. 5). These extensions (the lateral proximal lobes of Parker) have not yet grown caudally to surround the stalk, nor have they fused in front of the stalk. They extend along either side of the anterior dorsal portion of the anterior lobe (anterior half of the distal lobe of Parker) for quite some distance. The anterior lobe is very thick, and connective tissue infiltration has broken the lobe into nests of undifferentiated epithelial cells surrounded by blood sinuses. The neural lobe retains its lumen which is lined by an ependymal layer not at all different in appearance from that of the diencephalon. The neural lobe is directed rather sharply in the posterior direc-

tion and has made only a shallow dent in the roof of the anterior lobe. The intermediate lobe is much thicker than it is in the adult, being from two to five cell layers in thickness and very irregular in outline; it does not lie in juxtaposition with the posterior lobe as in the adult (fig. 6).

The cells of the anterior lobe, intermediate lobe, and pars tuberalis are entirely undifferentiated. Most of the cells stain a faint blue, but contain very little cytoplasm; no granules are seen. Although some of the cells take a rather deep blue stain and hint at being basophiles, they have been taken to be chromophobes, since they occur in all of the lobes of epithelial origin and because mitotic figures are seen in them at times. Thus, they could not be differentiated cells, since differentiated cells do not divide (Weiss, '39).

11th day. The lateral proximal lobes have fused anteriorly to form a single sheet of pars tuberalis. Posteriorly, in the region of the anterior lobe, these lateral proximal lobes are still separate and lie dorsally on either side of the anterior lobe as low, flattened, tubular bundles. These bundles are clearly separated by a strong layer of connective tissue. Beginnings of connective tissue infiltration into the posterior lobe are evident for the first time, as are signs of cellular differentiation in the anterior lobe. Small blue granular cells appear, as well as vacuolated blue cells; these basophiles are the first cells of the anterior lobe to differentiate. They represent about 12% of the cells counted. There are no signs of acidophilic granules in any of the cells, nor are any of the cells red in color.

18th-20th day. The distal lobe has completed its encirclement of the infundibular process which still has a wide lumen (fig. 9). The lateral proximal lobes are still tubular, but have not yet penetrated to the caudal side of the stalk. The intermediate lobe is a very thin epithelium, one cell layer in thickness. Basophiles are well developed, stain deeply, and represent about 18% of the cells. A very few, extremely dark red granular cells are seen (3%); these are the first acidophiles. These cells are small and elongated with but little

cytoplasm, usually with the nucleus at one pole and the cytoplasm concentrated at the other pole.

36th day. For the first time the pars tuberalis is seen to have completed its encirclement of the infundibular stalk, and is now seen caudal to as well as anterior to the stalk. Many of the nests of tuberalis cells still retain a central lumen. The connective tissue infiltration of the infundibular process which was first seen in the 23-day animal is well advanced at this time; this is the first evidence of a blood supply in the neural lobe. Vacuolated basophiles containing colloid are abundant in the anterior lobe, many of them present negative Golgi. Basophile percentage is 17%. Acidophiles are still seen, but rarely (8%) and indistinctly.

79th, 83rd, 85th day. The lumen of the posterior lobe has completely disappeared; thus, for the first time the gland is the morphological equivalent of the adult. The only differences which exist between these glands and that of the adult are those of the relative number of the different cell types, size of the glands, and size of the cells. Acidophiles, 22%; basophiles, 44%.

102nd, 104th day. In cytological appearance these glands are different from any others which have been examined, and their differences from the normal adult are more extreme than those exhibited following the longest periods of castration. The most prominent feature of the cells of the anterior lobe of immature opossums around 100 days of age is the tremendous amounts of colloid which fill the basophiles; basophiles are the most numerous of all of the cell types, being from 60 to 65% of the total number of cells counted. These colloid-filled basophiles (fig. 15) are swollen tremendously, some of them reaching the colossal diameter of 54 micra; an average of twenty-five of the larger ones coming to 36 micra. The colloid in these largest of all the cells yet seen in the opossum pituitary, stains differentially light to dark blue, indicating different phases of dispersion. Trabeculae of cytoplasm traverse the colloid. Only a small patch of cytoplasm exists, surrounding the nucleus and containing the greatly

enlarged and tortuous negative Golgi. The color of the ground substance of this fine-grained cytoplasm is a light pink; no colored granules are seen in it, although large, red hyaline bodies are scattered along the trabeculae, the cell membrane, and occasionally in the colloid itself (fig. 15). These hyaline droplets were described by Severinghaus ('39) as being typical of rat thyroidectomy cells after Bouin fixation; all of the glands of 102-104 days were fixed in formalin chrome sublimate. These colloid-filled cells are present in all sizes, from medium to very large, and contain varying amounts of colloid. Some of the largest ones are filled just short of the "bursting" point, but many show signs of accumulation or loss of colloid by having a cytoplasm incompletely vacuolated, or containing one to many droplets of colloid.

Granular basophiles are present as swollen cells containing distinct dark purple or blue granules; these cells do not contain colloid.

The nuclei of all of the basophiles show enlargement and stain deeply. Some of the nuclei show what would at first sight be called degenerative changes, such as pyknosis and vacuolation. These nuclear vacuoles are filled with a substance which stains a homogeneous blue, as does colloid. The negative Golgi areas are extremely tortuous and large, indicating accelerated liberation of secretion (excretion) according to Severinghaus ('39), or active elaboration of secretory products according to Bowen ('29).

The acidophiles in these unique pituitary glands exhibit large, dark red granules which only occasionally fill the cytoplasm. The appearance of the acidophiles in the sections indicates that they are squeezed out of shape by the greatly swollen basophiles. A few of the acidophiles show colloid vacuoles. Chromophobes are not unusual as to appearance, abundance, or scarcity.

It is an interesting fact that these appearances are shown by both normal males and females at this age, and also by members of both sexes which have been gonadectomized since

the twentieth to the twenty-sixth day, and further, that this colloid engorgement of the basophiles is not seen in opossum pituitaries of animals 2 weeks younger or 3 weeks older.

Cytological evidence in these glands indicates that these cells are in a storage phase (as evidenced by the colloid swollen cells), and are also actively elaborating secretion (as evidenced by the condition of the negative Golgi). The resemblance of 100-day pituitaries to a typical castration picture is very striking.

125 days. The great amounts of colloid and the greatly distended and swollen basophiles are no longer present in normal males of 125 days of age; the basophiles appear normal both in size and structure (fig. 16). In females of 125 days the number of these large basophiles is less than at 100 days, but not as low as in the 125-day males. Acidophiles are of the normal adult type in size and granulation, some of them tending to show droplets of colloid in the cytoplasm. The negative Golgi of the chromophiles suggest that the gland is in an active secretory phase and also an excretory phase, in contrast to the secretory and storage phase at 100 days. Many very large, clear cells are present which look much like degranulated cells; they are much larger than chromophobes. The presence of these cells seems to indicate that they might be the remainders of the large colloid-filled cells present around 100 days, after they had lost their stored secretory products and before they had time to return to normal chromophobe size.

150 days. Two of the three glands examined were from 5-month females, and one was from a 5-month male. They showed no striking differences from the condition seen in the 125-day males and females. The cells tend to be smaller than those of the adult, and the gland itself is much smaller than adult size. Basophiles are slightly more numerous than at 125 days in both males and females, and appear to be approaching the normal adult proportions.

V. THE RELATIVE PERCENTAGES OF THE DIFFERENT CELL TYPES

This section is divided into two parts; one is concerned with the pituitaries of adult male and female opossums, while the other deals with the developing pituitary of immature males and females. The observed trends and cell percentages of eleven adults, six males and five females, and thirty-three immature animals, eleven males and twenty-two females, are presented.

A. Adults

The six adult normal males used for cytological study of the pituitary were sacrificed during the months of January, April, June, and July; all of these males showed many active sperm in the epididymis, indicating they were in active sexual condition.

Male opossum pituitaries exhibited cell proportions of the following percentages: when taken as an average, acidophiles, 39.71%; basophiles, 44.08%; chromophobes, 16.13%. The range of variation within the acidophiles was 31.80–48.22%; basophiles, 21.49–52.30%; chromophobes, 7.52–36.80%. It is difficult to explain the great variations in the acidophiles and chromophobes, but a very probable reason for the basophilic variation seems to be that in those glands with the lower percentages of basophiles, large numbers of large, clear, agranular cells with typical basophilic negative Golgi were observed. It has been the practice in this investigation to classify such cells as chromophobes, since they exhibit no colored granules or cytoplasm; but it is believed that they are basophiles which have been depleted of secretory products.

Since all of the present information indicates that the male opossum does not undergo seasonal differences in sexual activity, it can be assumed that all of the males used in this study are comparable with respect to sexual condition. The female, on the other hand, shows a definite breeding cycle of a seasonal nature, coming into sexual activity in January and returning to the non-breeding or anestrus condition in June

and July. From the dates of the sacrificing of the adult females considered here, it is apparent that material from the two physiological states was examined. Of the five adult normal females, four were in breeding condition and one was in the anestrus state. For this reason, the two classes of females are treated separately.

The females in breeding condition showed the following average percentages of cell types: acidophiles, 32.36%; basophiles, 48.36%; chromophobes, 19.28%. The range in variation was: acidophiles, 27.35–37.48%; basophiles, 46.69–50.40%; chromophobes, 12.12–25.16%.

Since all of the percentages of cell types found in the breeding female fall within the range exhibited by the adult male, it is impossible to make a statement from this material as to whether sex differences exist in the pituitaries of breeding male and female opossums.

It is unfortunate that only one female in the anestrus period is available at this time, for its differences from the pituitary of the breeding female are very striking (fig. 14). The acidophiles in this pituitary were found to represent 27.11% of the total number of cells counted; basophiles, 19.92%; chromophobes, 52.96%.

Within the cell types certain tendencies are apparent. Dark acidophiles were found to be more abundant than light acidophiles in all individuals except one male and one female; and the average of dark acidophiles was greater than that of light acidophiles in both sexes. Basophiles may be said to be the chief cells of the adult opossum pituitary during the breeding season, and within the basophiles the non-granular basophiles are by far the most frequently seen type. The colloid-containing basophiles are the next most abundant, while the granular basophiles are always the least numerous.

The most striking shift in percentages of cells which takes place in the adult female opossum pituitary in the change from the breeding to the non-breeding condition is the great decrease in basophiles with a corresponding increase in the number of chromophobes. The acidophiles apparently are the

same in number and appearance in the specimens studied, thus it is not surprising that these effects should be most noticeable in the extreme anterior end of the gland, that is, in the basophilic region.

B. Immature animals

The most prominent cell type present in the pituitary glands of the immature opossums of less than 80 days is the chromophobe. From day 1 to day 8 all of the cells in the anterior lobe are undifferentiated, and were therefore classed as chromophobes. From day 11, when the first differentiated cells are seen (basophiles), through day 54, the number of chromophobes decreases as the number of differentiated cells (chromophiles) increases. The chromophobic percentage in the animals of 83–150 days is either less than or the same as the percentage of chromophiles; but it is usually less. Thus, the percentage of chromophobes decreases from birth through 150 days; and is always less than the chromophilic percentage in the breeding adults.

Basophiles are the most prominent cells in the adult breeding condition; they are also the most abundant of the chromophilic cells in the immature gland. From the time they are first seen at 11 days through 150 days, they are present in greater numbers than acidophiles. Usually their edge in numbers is quite pronounced, particularly around 100 days and 150 days.

Of the types of basophiles, the granular ones are least numerous; non-granular, next most numerous; and colloid-containing basophiles, most numerous. The colloid-basophiles remain fairly constant from day 23 to day 54, around 10%. Beginning on day 54, a rise in numbers is seen which ascends rapidly as judged by glands at 83–85 days which show the colloid-basophiles to be about 34%. By 93 days the rise has reached 42%, and at 102 days, reaches the peak of 54%; this is followed by a rapid decline during the subsequent 3 weeks, so that at 125 days, the percentage of colloid-basophiles has dropped to 28%. At 150 days the percentage of 16% is prac-

tically that of the adult (male — 12.95%; female — 15.64%) adult percentages.

From the results of the cell counts of immature opossum pituitaries, the acidophilic percentages show the most variation. The only generalization that can be made is that they are usually less abundant than either basophiles or chromophobes up to day 125. By day 150 the acidophiles are present in the anterior lobe in about the same numbers as in the adult — 38%.

Within the group, acidophiles, the two types — light and dark — show fairly uniform percentages of occurrence. From the day when they are first seen (18 days in the present material) to day 54, the light acidophiles are more numerous than the dark. From day 54 to day 150 the dark acidophiles are the most numerous; and at 150 days, the two types were found to be present in about equal numbers (14%).

Considering the possibility of sex differences, no regularities which could permit a positive answer were observed. However, differences in the percentages of basophiles between male and female glands subsequent to the hundredth day show that from this time until 150 days, the female anterior pituitary contains greater numbers of basophiles.

VI. THE EFFECTS OF GONADECTOMY DURING DEVELOPMENT

This series deals with a total of thirteen immature opossums (nine males, four females) which were castrated while still in the pouch between day 20 and day 40, and left for periods of time varying from 32 to 76 days. The question which presents itself is this: What effect does gonadectomy have on the histological picture of the anterior pituitary of the opossum which is undergoing sexual differentiation?

Histological examination of the pituitaries of both normal and gonadectomized opossums of the same or corresponding ages reveals no striking differences except in the youngest stages, 52–54 days. The very obvious differences in the 52- to 54-day animals are the differences in the number of basophiles, especially the colloid-containing basophiles. The normal 54-

day male shows a cell count percentage of about 13% colloid-basophiles, while the castrate male of 52 days (gonadectomized from day 20-52) has 22% colloid-basophiles. The females of this age group show even a greater divergence; the normal 54-day female has 9% colloid-basophiles, while the 52-day gonadectomized female (ovariectomized from day 20-52) has 32% colloid containing basophiles. These differences are quite plain even with a casual examination of the sections.

As for the other age groups, no significant differences are seen in the pituitaries of the normal and gonadectomized animals. The normal and gonadectomized specimens show approximately the same percentages of colloid-basophiles at 83-85 days, while at 102 days, the normals show slightly greater colloid-basophile percentages than do their normal controls.

The pituitary of a male gonadectomized on day 22 and sacrificed on day 140 shows very striking differences from a control of the same age. The castrate male shows the tremendous accumulation of colloid-basophiles so typical of castration and of normal 100-day animals. Cell counts show the percentages of colloid-basophiles to be about the same as at 100 days, but the cells themselves are not swollen as they are at that time.

Up to 104 days no differences which could be correlated with sex or the length of castration period were noticed. Thus, except for the great differences apparent at 52-54 days, gonadectomy during development (up to 104 days) seems to have no noticeable effect on the pituitary gland, which is also undergoing development. Subsequent to day 104 (on day 140), typical castration differences were observed between a control and gonadectomized pair of males.

VII. THE EFFECTS OF HORMONE ADMINISTRATION

Meyer, Leonard, Hisaw, and Martin ('30, '31, '32), Moore and Price ('32) demonstrated that estrogens injected into normal adult rats reduced the sex-stimulating potency of their pituitaries. Leonard ('33) reasoned that if this effect was

due to estrogens, then substances which increase the estrogen output of the ovary (such as PU extracts or other gonadotropins) should also decrease the potency of the implanted pituitary. He injected rats with PU extract and proved his point, and further showed that the PU extract had no effect on the pituitary of a gonadectomized animal. These results stimulated Wolfe, Phelps, and Cleveland ('33), Severinghaus ('34 a, b), Wolfe, Ellison, and Rosenfeld ('34 a, b, c), and Wolfe ('34) to determine what cytological changes in the pituitaries of these injected animals could be correlated with the loss of gonad-stimulating potency. These studies revealed that the pituitaries of the injected animals were markedly altered from their controls. In general, the results of these studies agree as far as the description of what the changes are. The effects noted were: (1) increase in size of basophiles and marked loss of granules; (2) the acidophiles, many of which were swollen, also showed evidence of granular loss; (3) cell counts revealed that the percentage of acidophiles was decreased, basophiles increased, and the percentage of chromophobes was increased; (4) marked increase in size of the Golgi apparatus, seen in positive or negative image.

Some thirty-eight glands make up the material which is being reported here; most of the animals have been treated with gonadotropins (13 adult females, 5 immature females, and 5 immature males), while relatively few are reported here which received sex hormones (estrogens: 2 adult females, 2 immature females, 3 immature males; androgens: 2 adult females, 2 immature females, 2 immature males).

A. Gonadotropins

1. *Adult series.* For convenience, the adult series of opossums which received treatments of gonadotropins will be considered first. The dosages varied from $\frac{1}{2}$ RU per day to 10 RU per day, and the total RU received varied from $3\frac{1}{2}$ to 200 RU. Since all of these treatments were carried out on adult females during the anestrus period, the control used for comparison is a normal anestrus female. The effects

observed show a tendency to be more marked in the animals which received the greatest dosages, but the individual variation is great enough in normal animals to necessitate large series of animals to verify this suggestion.

The most striking effect of gonadotropin treatment to be observed in the pituitary glands of adult anestrus opossum females is the great number of large degranulated cells, correlated with an increase in chromophobes and a decrease in non-granular basophiles and dark acidophiles. The cytoplasm of these large clear cells is finely granular and takes a gray to light pink color. The nucleus is large, usually vesicular and contains large chromatin particles at the periphery. Because of the large size of these degranulate cells, and because they are seen at times to contain small amounts of colloid, they are considered to be of basophilic origin. However, they are considered as chromophobes in the cell counts. Another striking effect of treatment with pituitary gonad-stimulating hormone is the great increase in the number of large granular basophiles. It was noted earlier in this paper that granular basophiles occur but rarely in the normal adult opossum pituitary. The percentage of granular basophiles in the normal control is about 1%, while in the gonadotropin treated females, this percentage reached 6% and 7%, an increase of over 600%. These basophiles display large, spherical, dark blue to purple granules, and the cells themselves appear to be swollen. At times, a cell is seen which appears to be bi-granulate, in other words, to have a mixture of both red and blue granules, with the majority of the granules being blue. The colloid-basophiles are present in the pituitaries of the treated animals in about the same percentage as in the normal control and appear to be normal in all respects.

Acidophiles in the injected animals undergo varying amounts of reduction in number, and almost always exhibit large, distinct granules. Thus, it appears that along with a thinning out of granules, an increase in the size of the individual granules occurs. This effect is quite striking and very obvious. Too, the acidophiles appear more distinct because of

their reduction in numbers, that is to say, they occur in ones or twos rather than in large groups. The decrease in acidophiles is most effective in the dark acidophile type, and in Opossum Serial 126 A, treated adult female which shows the most extreme acidophilic decrease, the dark acidophiles are completely eliminated. In this female which received the largest total amount of gonadotropic substance and showed the most extreme effects of treatment, the total acidophile percentage (all light) was only 3.43%, a decrease of some 900% from the normal control.

Chromophobes are the most abundant cells of both the treated and the normal control animals, but differences appear in the treated specimens. The chromophobes of the injected females are unique in having distinct cell outlines and cytoplasm so pale that it is hard to tell if any is present; the nuclei are densely chromatic. Another difference seen in the chromophobes of the treated females is their occurrence in huge nests or "islands." This is quite different from the normal gland where the chromophobes appear as interstitial cells packed between the chromophiles.

From examination of the serial sections it is readily seen that small doses given over a long period are more effective in producing changes in the cells of the anterior lobe of the opossum pituitary than are large doses given for a short period.

2. Immature series. Ten immature opossums, five males and five females, ranging in age from 70 days to 150 days make up the material studied in this section.

Since Leonard ('33) and others have shown that gonadotropins injected into gonadectomized animals are without effect on the pituitary gland of such animals, it is one of the objects of this part of the present investigation to determine when in terms of hypophyseal cytology, the gonads become receptive to anterior pituitary stimulation. The data collected thus far seem to indicate no response prior to day 79 and a slight response of the pituitary of 79-day males following gonadotropin treatment. The indication is based on a reduc-

tion in the number of basophiles (particularly colloid-basophiles) and a corresponding increase in the percentage of chromophobes. No striking changes are noticeable in the structure of the cells of the treated animals. The one 79-day treated female however, does not show effects of treatment either in cell structure or in shift in cell proportion.

Summarizing the effect of treatment shown by 70- to 79-day animals, it can be said that definite signs in the 79-day male suggest that the gonad at this time is responsive at least to some slight degree to stimulation by gonadotropic substance.

One male and two females, 125 days old, which had received a total of 65 RU of gonadotropin in 12 days, make up the material for the next stage. It must be remembered that at 125 days the pituitary shows the great effect of loss of stored colloid present at 100 days, and for this reason, normally gives the appearance of a somewhat depleted gland. This normal depleted effect is much more noticeable in the male than in the female at 125 days. Study of the sections of this material shows the greatest effect of treatment at 125 days by the females, while the male shows but little noticeable effect. Cell counts show, however, that some effect is exhibited by the treated 125-day male. In the female at this age the changes are of the same nature as those shown by the adult females, but not so extreme. The basophiles are largely degranulated, but many colloid-basophiles remain. The acidophiles show some tendency to exhibit large, sparse granules, but not as greatly as do the treated adult females; many acidophiles are present which appear to be homogeneous and non-granular in nature. It is curious to note that the treated females greatly resemble the normal males of the same age. This is a further suggestion that at 125 days the normal male is secreting androgen in more effective concentrations than the normal female is secreting estrogen.

B. Androgens and estrogens

Wolfe and Hamilton ('37) showed that 500 gamma of testosterone propionate daily for 10 days would degranulate the

basophiles in the anterior lobe of immature male and female rats. This effect is similar to the injection of estrogens into normal animals (Severinghaus, '34 a, b).

Two adult females, two immature males, and two immature females make up the material which was studied for the effects of androgens on the pituitary gland of the opossum. Four adult females, two immature females, and three immature males were studied for the effects of estrogens on the hypophysis.

1. *Adult females.* Of the two animals available for this study, which were treated with androgens, one received 10 mg. of testosterone propionate in 10 days, while the other received but one injection of 2 and $\frac{1}{2}$ mg. of testosterone propionate. The effects were noticed only in Opossum Serial 121, the female which received 10 mg. testosterone propionate. Two of the estrogen treated females were anestrus animals, while the other two were anestrus females which had been spayed 30 days previous to autopsy. Estrogen treated females received 10 RU Progynon-B per day for 12 days. A great increase in the number of chromophobes was one of the effects of these injections, as well as a decrease in the numbers of acidophiles. The basophiles exhibit no great shifts in actual numbers, but show different percentages of the various types of basophiles; the greatest shift is the increase in the number of granular basophiles with a consequent decrease in the numbers of non-granular basophiles. The numbers of colloid-containing basophiles remain unchanged. These shifts in numbers greatly resemble those exhibited by the adult females which were treated with gonadotropins. The acidophiles exhibit large, sparse granules, while great numbers of degranulated cells are seen. No effects which could be called degenerate were observed. The effects of Progynon-B in the females show that not only were castration changes prevented by this treatment, but also that the doses were greater than those produced by the ovary either during the breeding or non-breeding season.

2. *Immature animals.* The object of this incomplete survey is to determine whether gonadal hormones are capable of effecting changes in the anterior pituitary of opossums which are undergoing development. The eight animals considered range in age from 30 days to 93 days, and include four males and four females.

The results are not at all uniform in the younger animals. No effect at all can be seen in Opossum Serial 108 and 109, the 30-day male and female which had been treated with estradiol ointment from day 6-29. Opossum Serial 64 A and 64 B, 31-day male and female which received testosterone propionate treatment from day 10-30, on the other hand, show a decided degranulation effect. There are very few acidophiles and basophiles present in these glands, but no large degranulated cells are present as in the hormone treated adults. Opossum Serial 110, 43-day female, Opossum 111 and Opossum 115, 46-day females treated with estradiol ointment, show great increases in the number of granular basophiles, but a decrease in the total number of basophiles. These changes are similar to those noted in adults which have been treated with hormones. These results tend to indicate that the pituitary of 30- to 31-day opossums is capable of responding to testosterone propionate, but not to estradiol ointment, and that the pituitaries of 46-day females will respond to estradiol by decrease in basophiles.

The oldest immature animals treated with testosterone propionate show quite typical changes and make it unquestionable that at this age (93 days) the pituitary of the male opossum at least, responds to treatment with this hormone. The increase in granular basophiles is seen clearly by a casual examination of the serial sections, as well as the decrease in the numbers of non-granular and colloid-containing basophiles and the increase in chromophobes. It should be remembered that at 93 days, the normal opossum pituitary is heavily laden with large, colloid-swollen basophiles, and presents the general impression of long term castration. The

contrast of the treated males with the controls is exceptionally great, since the greatest effect of the hormone is to cause the disappearance of great amounts of this stored colloid. The resulting picture is similar to that of the 125-day males, in which most of the colloid has disappeared following the beginning of liberation of hormone from the testes of the normal male.

VIII. DISCUSSION

A. Castration changes in the adult

The changes in the adult opossum pituitary following gonadectomy conform well with those described for the majority of other species. This investigation has shown that castration cells do develop in the anterior hypophysis of the adult opossum which has been gonadectomized, that the number and size of the basophiles, particularly the colloid-filled basophiles, show decided increases, and that the number of dark (heavily granulated) acidophiles decreases. It was pointed out that colloid-filled and vacuolated basophiles occur in all normal opossum pituitaries and that these cells are not similar to castration cells since they have a nucleus which is normal in all respects and not pyknotic, and the colloid pools are usually small; never do they crowd the nucleus to one pole of the cell. Vacuoles, when they do occur in opossum basophiles, appear in the pool of colloid. Vacuolated basophiles in normal glands have been reported for birds (Payne, '40) and for amphibians (Applington, '42). Severinghaus ('39) presents an illustration of a basophile in the pituitary of the human taken at the end of a normal pregnancy which contains what he terms "a characteristic cytoplasmic vacuole." It is not known that colloid-containing cells have ever before been reported in normal pituitary glands of mammals.

The observed changes in the opossum pituitary following removal of the gonads are consistent with the reports on the pituitaries of other forms and tend to lend support to the mass of evidence associating the basophiles with the production of gonad-stimulating hormone.

B. Cytogenesis

The process of cellular differentiation of the mammalian hypophysis has been studied in man, Fraser ('21) and Cooper ('25); in the pig, Nelson ('33); in the cat, Brahms ('32); and in the horse, Harrison and Shryock ('40). In all of these forms differentiation of the cells of the anterior lobe takes place in the fetus. Since the marsupial has such a brief intra-uterine existence, it should be expected that the processes of cytogenesis might not occur until the young are in the pouch. This assumption is justly made, since this investigation has shown that the earliest signs of differentiated cells, basophiles, appear in the anterior lobe of the 11-day pouch young and not in the 8-day pouch young, whereas the first acidophiles are seen in the 18-day pouch young and not in the 11- or 13-day pouch young.

The present investigation has also shown that by the time the first acidophiles appear, the basophiles are well established and fairly abundant. In human material Cooper ('25) found acidophiles by the end of the third month of intrauterine life, and basophiles by the end of the fourth month. In the cat Brahms ('32) noted cellular differentiation in the 75-mm. fetus, but he did not specify which types of cells he saw. Harrison and Shryock ('40), working with horse material, found that acidophilic granules appear in the cytoplasm of cells in the pars distalis of a 64-mm. fetus, and not in a 34-mm. specimen. They also found the first definitely recognizable basophilic granules in a 138-mm. specimen. In the pig Nelson ('33) reports basophiles in anterior lobe tissue from 50-mm. to 60-mm. fetuses, and acidophiles in tissues from 70-mm. to 80-mm. fetuses. Thus, in the pig and the opossum, basophiles appear before acidophiles, while in man and in the horse, acidophiles differentiate before basophiles. Parker ('17) made a few observations on the differentiation of cells in the marsupial anterior lobe while investigating the embryology of the hypophysis of these forms. The stages at which cellular differentiation was observed by Parker were as follows:

Trichosurus (11-mm. head length), *Phascolarctos* (9.5-mm. head length), and *Dasyurus* (13.5-mm. neck-rump length). An opossum pouch young with a head length of 11 mm. is about 16 days old; 9.5-mm. head length, 12 days; and 13.5-mm. neck-rump length, 10–11 days. From the data which Parker gives, if the measurements are at all comparable, the Australian marsupials which she studied and the American opossum compare well as to time of cellular differentiation of the anterior hypophysis.

The one great difficulty in establishing the exact time of cellular differentiation is that the development of specific granules or of particular cell types is not a sudden process but a gradual one. This seems to be especially true of the acidophiles, in fact so much so, that suggestions of acidophilic differentiation were noticed in specimens slightly younger than those in which typical acidophiles occur.

The peculiar phenomenon suggesting storage, which is exhibited by the pituitaries of the animals of 102–104 days, is a difficult problem to approach. It has been mentioned before that this condition is not seen in glands from animals 2 weeks younger or 3 weeks older, and is present alike in both sexes, both normal and gonadectomized. The condition is perhaps foreshadowed in glands from 83- to 93-day animals. In these glands colloid-basophiles of the same general type are seen, but are not enlarged or swollen to the exaggerated dimensions displayed at 100 days; they are also quite numerous. The fact that this condition is exhibited by normal as well as gonadectomized animals tells nothing, for this sort of thing could be the result of the precocious differentiation of the pituitary at a time when the gonads had not yet begun to liberate sex hormones which could prevent this tremendous accumulation of colloid. Another suggestion is that the pituitary cells have become differentiated and have stored secretion, but have not yet reached the stage at which their secretory products are liberated. This condition would be similar to that of the pituitary in the rat, or of the thyroid in amphibia. It has been known for a long time that in each of these animals these

glands contain secretory products which, if liberated in another animal of the same age by being absorbed following implantation, produce definite effects which these glands do not affect if left intact in the normal animal. These facts have been interpreted to indicate that the gland has elaborated and stored the secretion, but is either not able to liberate the secretion, or the proper stimulation for secretion is lacking. On the other hand, it can not be determined with the present data whether this phenomenon is the result of reproductive activity, since the pituitary is known to affect so many other systems, both endocrine and non-endocrine. In other words, this cytological picture may be found to be correlated with thyroid physiology, or with any number of other things. To be certain within reasonable doubt that this picture and its subsequent disappearance in the later stages is not to be correlated with the gonadal activity, pituitaries from animals of 125-150 days of age which had been gonadectomized prior to day 100 must be studied. If it were found that in the gonadectomized animal of 125-140 days the colloid storage still remained, it would appear that its disappearance from the anterior pituitaries of the normal animals was due to the presence of gonads, since this would be the only way in which these two animals differ. However, if the colloid had also disappeared from the pituitary of the gonadectomized animal of 125-140 days as it has in the normal, it would be apparent that the presence of gonads in the normal animals was not the influential factor causing its disappearance. Since the colloid still remained in a 140-day male castrated from day 22, it is very probable that the gonads are the influential factor causing the disappearance of colloid in normal animals subsequent to day 100.

Nothing at all is known about the chemical nature of the colloid in the swollen basophiles, whether it is the actual stored secretory product, or whether it is capable of effecting gonad stimulation by implantation. Therefore, it is imperative that implantation experiments be performed when the material becomes available in order to test these points.

C. Relative percentages of different cell types present in adult and juvenile pituitaries

1. *Adults.* Structural differences in the anterior lobe of male and female mammal pituitaries have been reported for the rat, McQueen-Williams ('34), Wolfe and Ellison ('34); and for man by Rasmussen ('29, '33); these differences are based on numbers and staining reactions of the cells. Kirkman ('37), after a thorough study of the adult guinea pig hypophysis, presented evidence which indicates that in this animal, a significant sex difference does not exist. Seasonal differences based on structural differences have been reported for the woodchuck by Rasmussen ('21); lizards, Altland ('39) and Poris ('41); amphibia by Zahl ('37) and Applington ('42); fish, Bock ('28), Mathews ('36). In all of these studies a maximum of activity is displayed during and just preceding the breeding season. That is, a greater number of chromophiles is present, which in most cases is basophiles.

In the present investigation the information available does not indicate a difference in the sexes during the season of reproductive activity; this is because all of the percentages of cells fall within the same range of variation. However, the female pituitary does exhibit seasonal changes correlated with lack of reproductive activity, or at least with a greatly reduced activity of the hypophysis. The most significant change seen in the anestrus pituitary is a decreased number of basophiles, and a corresponding increase in chromophobes. This is interpreted as indicating a resting gland. It has been shown by Morgan ('42) that the anestrus pituitary which was examined, actually was releasing gonad-stimulating hormone. In November one ovary of this animal was removed under sodium amytal anaesthesia by surgical means. This ovary was weighed and sectioned for histological details of structure; the other ovary was removed 1 month later and was found to have increased greatly in size and weight. The size and weight of an ovary are largely a function of the number of follicles which it contains. Thus, this hypertrophied

ovary must have been stimulated by an anestrous pituitary. Since the pituitary concerned here was found to contain about 20% basophiles, it is likely that their presence could account for the observed activity.

2. *Immature animals.* Halpern ('38) reports that differential cell counts of human fetal and post fetal pituitaries show a greater percentage of chromophobes in the fetal gland. The present study has also shown a greater percentage of chromophobes in the immature opossum pituitary; this advantage in numbers becomes less and less with increase in age, until at 150 days the normal adult percentage is approached. The cytological picture of the undifferentiated cells in the immature animal is not unlike that of the chromophobes of the adult; this is evidence that the chromophobes are resting cells which upon activation, differentiate granules, discharge these as secretion, and revert to the chromophobe stage. Looking at this phenomenon in another fashion, this could be called modulation in the same sense with which Weiss ('39) uses the term. Thus, reversion to the chromophobe state is not de-differentiation, rather it is one extreme of the cell's normal potentialities, and oscillations between this extreme and the other of granulation occur in the cycle of function of the cell.

Acidophiles show a curve increasing in slope from the time of their first appearance around 18 days to the approximate normal level at 150 days. It is thought that the minor oscillations in this curve are the result of normal individual variation and that with more material they would not be as great. The fact that the number of acidophiles in the very young forms is small and increases with age until the adult level is reached is further evidence that this is a distinct cell type and not one related to basophiles.

Basophilic changes in proportions follow the general tendency of acidophiles in showing a gradual increase in numbers from the time of their first appearance in the gland. The extreme basophilia exhibited by the pituitary at 100 days has been discussed under cytogenesis.

No evidence is available as to what time in the developmental history the chromophiles become potentially functional; and it is still another matter as to when these cells actually begin to release secretory products. The great mass of evidence indicates that the cells become potentially active at the time when there are granules present in them, but some more recent work on the developing chick hypophysis shows that melanophore expanding hormone is present before stainable cellular differentiation (Chen, Oldham, and Geiling, '40). Judging from differential staining reactions, the opossum pituitary is potentially capable of basophilic function subsequent to the eleventh day, and of both types of chromophilic function subsequent to the eighteenth day.

The tremendous basophilic increase up to 100 days followed by a great decrease in basophiles during the next 3 weeks indicates that a great release of secretory products has occurred between 100 and 125 days. It is known that this decrease in basophiles is related to the gonads, and a possible correlation is seen in the male, since this effect occurs in the normal animal and not in the castrate. According to Moore ('41 a), the testis at 100 days displays seminiferous tubules which consist of a single basal layer representing the germinal epithelium but not tubular lumina. Moore and Morgan ('42) describe the normal testis in the 150-day male as being decidedly different than at 100 days, in that the tubules at this time possess lumina and an actively developing germinal epithelium; Leydig cells are found in the intertubular areas. These facts suggest that the testicle at 150 days is actively producing androgens; therefore, the pituitary must have stimulated this androgenic activity. This is further emphasized when it is seen that in the male it is the colloid-basophiles which show the greatest decrease in numbers from day 100 to day 150. This is also evidence for considering the colloid-basophile as the storage phase of the secretory cycle. These changes in the pituitary of the female opossum are not as pronounced as they are in the male, but it is significant that a reduction in the number of basophiles does occur in the

female between 100 and 125 days. In the male the basophiles are reduced from about 60% at 102 days to 20% at 125 days, while in the female the reduction is only from 65% to 43%. Morgan ('42) reports that in the female of 100 days, no antra are present; by 125 days very small antra are present in some of the follicles. These remain until 150 days and are completely gone by the eighth month when the animals go into the anestrus period. The presence of antra is histological evidence of production of estrogenic substances. Since these are first seen at 125 days, it is possible that the partial reduction in numbers of basophiles in female pituitaries between 100 and 125 days is responsible for the appearance of these antra, or vice versa. Thus, if this reduction is due to release of stored gonadotropins, it should be expected that the reduction should occur first in the sex which showed the first signs of producing sex hormones; in the opossum it is the male. Further, a similar reduction in basophiles would be expected to occur at the time of antrum formation in the female. At 150 days, when the testis shows signs of endocrine activity and the ovary still contains antra, the total percentages of basophiles in male and female glands is about equal; the female shows about a 10% advantage in numbers which can not be accepted as significant because of the wide range of variation. At this time the number of colloid-basophiles is approximately twice as great in the female as in the male gland; male, 18%; female, 33%. The interpretation of this cytological evidence in terms of gonadal histology has a logical explanation. The male at 125 days shows the first signs of androgen elaboration; this is correlated with a tremendous decrease in numbers of basophiles. The testicle from this time on continues to secrete androgens and to produce sperm without the intervention of a resting period. In the female the first small antra are visible between 125-150 days, and their appearance comes at the same time as does a less radical reduction (than in the male) in the numbers of basophiles in the pituitary. By the eighth month, all of the antra are gone, indicating that the female goes into an anestrus period shortly following a partial awakening of

the ovary to endocrine activity. Since the beginnings of endocrinal function of the ovary are only temporary, the reduction of basophiles in the pituitary is not as pronounced as in the male.

D. The effects of gonadectomy during development

Clark ('35 a) found that from birth to 20 days, the anterior hypophysis of the female rat contained more gonad-stimulating potency than did the male; this is the reverse of the condition in the adult. Severinghaus ('35, '36) found an increase in the number of basophiles in the young female rat over the male prior to 20 days, thus correlating more basophiles with a greater gonad-stimulating potency and linking the basophiles to reproductive stimulating hormone. Further, these facts were interpreted as evidence that the ovary does not secrete sufficient estrogen prior to day 20 to affect the hypophysis, while the testis has already begun to secrete amounts of androgens sufficient to affect the anterior lobe cells. Thus, in the rat the testis begins to function before the ovary. This phenomenon received further proof when it was found by Severinghaus ('39) that male rats castrate from birth to 20 days have pituitaries which greatly resemble those of normal 20-day females; this resemblance even applies to tests for gonad-stimulating potency by the implant method (Clark, '35 b).

The results of this investigation have shown that except for animals of 52-54 days, no detectable differences are seen in pituitaries of normal and gonadectomized opossums up to 102 days of age. As to the time when gonad hormones are being secreted in sufficient amounts to affect the anterior pituitary of the developing opossum, it was concluded in another section of this paper that prior to the hundredth day, no evidence, either from the pituitary cytology or the effects of castration on the development of the accessory reproductive organs (Moore, '41 b), is known which would indicate that the gonads during this time are secreting detectable amounts

of sex hormones. Thus, since castration effects have only been found in other animals which had functional gonads, it is not surprising to find that gonadectomy during development in the opossum, whose gonads presumably have not yet begun to function as endocrine glands, is without effects apparent in the anterior pituitary.

The crucial test as to whether the gonads of opossums between the ages of 100–150 days are secreting detectable amounts of hormone concerns the animal which had been gonadectomized prior to this time and brought to autopsy at 140 days. It was found that this animal possessed a pituitary which still showed the excessive accumulation of colloid-containing basophiles so characteristic of 100-day opossums of both sexes, normal and gonadectomized, which disappear by the one hundredth and twenty-fifth day. Two suggestions can be made: (1) Prior to the hundredth day, the gonads of immature opossums have not yet begun to liberate sufficient amounts of sex hormones to affect the normal pituitary, and thus, removal of the gonads is without the usual castration changes. (2) Subsequent to the hundredth day, the gonads of the male have begun to secrete androgens in amounts capable of releasing the stored colloid in the basophiles of the anterior hypophysis, while in the female the ovary may have begun to secrete estrogens, but in not as great potencies as the male secretes androgens, since the effects present in the pituitaries of the two sexes are different — the female showing the least effect.

Concerning the difference between the pituitaries of normal and gonadectomized opossums of 52–54 days, the conditions need more evidence for explanation. No explanation for these differences is apparent; it may be that the normal animals of this age which were examined in this work are not typical, since they contain fewer chromophobes than do the pituitaries of animals 3–5 days younger, and the general tendency seems to be a relative increase in the percentage of chromophiles with increase in age. It is apparent from examining the sections of these normal animals of 52 days, one male and one

female, that they present the same cytological picture, which is evidence against their being atypical. At present, all that can be said is that more material and work needs to be done on specimens of this age.

E. Hormone treatments

1. *Gonadotropins*. The physiological evidence indicates either an inhibition of elaboration of secretion, or a lack of storage of secretory products in pituitaries of hormone treated rats (Meyer, Leonard, Hisaw, and Martin, '30, '31, '32; Moore and Price, '32). The cytological evidence from the pituitaries of these treated rats has been interpreted by Severinghaus ('34 a, b, '36, '37, '39) to indicate an excessive stimulation of the pituitary to rapid elaboration of secretion, with the consequent excretion of those products as rapidly as they are formed. The appearance of granular cells is quite rare in these pituitaries, since granules are universally thought to be the cytological evidence of elaborated glandular secretion (Bowen, '29).

The opossum is a unique mammal in many ways, one way is that it is the only form reported thus far to contain basophiles with colloid in them in the normal animal. It must be admitted that nothing is known at present about the chemical nature of this substance called a "colloid." It presents all of the cytological evidence of being colloid, and has been verified as such by Prof. R. R. Bensley. Further work is indicated to determine whether this colloid is actually stored secretion. Thyroid follicular colloid has been isolated and has been shown to contain the active principle of thyroid secretion. If it is found that a correlation exists between gonad-stimulating potency and the number of colloid-containing basophiles in the anterior lobe of the opossum, we can then feel certain that this colloid represents stored secretory products.

It is true that the adult opossum pituitary and the immature opossum pituitary (79-day male and 125-day male and female) respond to gonadotropin by degranulation. In the

125-day animals this hormone causes a decided decrease in the numbers of colloid-basophiles; but in the adult anestrus female treatment affects colloid-basophiles but very little, if at all. Thus, if this colloid is stored secretion, and Severinghaus is correct in saying that hormones stimulate rapid and increased liberation of secretion, it should be expected that this colloid would be one of the first results of hormone treatment. It can be seen that this is just what happens in 79-day males and 125-day males and females, but not in the adult anestrus female, where the greatest basophilic change is an increase in the number of granular basophiles. No ready explanation of this problem presents itself at this time.

2. *Androgens and estrogens.* The effects of gonadal hormones are similar to those following gonadotropin treatment. The reason for separating these two treatments is because it has been found that in the earlier stages the opossum does not respond to treatment with gonadotropin (Moore, '41 a).

The lack of uniformity of effects of sex hormones on the pituitaries of very young opossums is difficult to explain, since so little information is available at present. All that can be said is that with the few animals observed, testosterone propionate effected a typical adult-like degranulation in a 30-day male and a 30-day female, while estrogens caused no visible or statistical effects in a 31-day male and female. More material and intermediate stages with similar age controls might reveal the fact that estrogens as well as testosterone propionate are capable of influencing the 30-day opossum pituitary. At 43-46 days estrogen treatment from day 10 on is effective in inducing hypophyseal changes of the same sort as those which occur in estrogen treated adults. This is indication that although the gonads are not secreting at this time and are not susceptible to gonadotropic stimulation, the hypophysis is partially differentiated and the differentiated cells are sensitive to gonadal hormones. It is also possible that the differentiated cells in the pituitary at this time are liberating hormone, but since the gonads are not capable of responding, no visible effects can be seen.

The effects of testosterone propionate on the 93-day animals is very significant. It was stated earlier that at 93 days the normal pituitary is crowded with small basophiles — most of them containing colloid. In other words, at 93 days it is possible to foresee the climax condition reached at 100 days of extreme basophilia. These glands of 93 and 100 days give the impression of long term castration and probably are the result of the pituitary differentiating prior to the time the gonads have differentiated to the point of liberating sex hormones. This seems to be fact from the evidence gained from the testosterone propionate treatment of the 93-day males. In these treated males the colloid-basophiles are reduced to one-half the numbers shown in a 93-day female control; the non-granular basophiles are reduced and the granular basophiles are increased in number. The resulting picture is similar in many ways to that of the normal 125-day male, where the depletion of the colloid seen at 100 days is almost complete. These findings appear to be sufficient evidence to conclude that with the material studied, the pituitary of the immature opossum differentiates long before the gonads do, and as a result, conditions similar to castration changes are shown up to the time when the gonads begin to function as endocrine glands. The male, subsequent to inception of endocrine function, continues to secrete male hormone and the pituitary assumes the adult structural condition. The female, after a partial awakening of the ovary to endocrine function, soon goes into anestrus and, thus, the pituitary does not assume the adult picture until the first breeding season.

IX. SUMMARY

A. The adult opossum pituitary

The pituitary gland of mature opossums contains all of the usual epithelial and neural components.

Basophiles of three distinct types make up the chief cells. Acidophiles are not unusual in appearance and all stages of granulation from sparse to complete are seen in all glands.

Chromophobes are the least numerous and the most inconspicuous cells of the adult gland. Regional distribution of the cell types exists so that the basophiles and chromophobes are most numerous in the anterior portions and decrease posteriorly, while the acidophiles are confined mostly to the posterior portions and decrease anteriorly.

B. Effects of adult castration

Gonadectomy of periods from 30 to 136 days causes extreme basophilia of the adult gland. In the longer periods the effects are more extreme than in the short periods. Castration cells occur in glands from animals castrate for 60 days or more.

C. Normal development and cellular differentiation

1st day. At birth the lateral lobes (anlagen of the pars tuberalis) are separate, and the anterior and intermediate lobes form a shallow sac which is only slightly indented by the hollow down-growing neural lobe. All of the cells of epithelial origin are undifferentiated.

11th day. The first fully differentiated cells to appear are seen to be basophiles; no acidophiles are present.

18th-20th day. Acidophiles make their first appearance at this time.

By the forty-second and forty-third day the pars tuberalis is no longer tubular, and by the seventy-ninth day the lumen of the posterior lobe has completely disappeared. Thus, at this time the gland is the morphological equivalent of the adult except for size and relative proportions of cell types.

D. The relative percentages of cell types

1. Adults. Males exhibited the following average percentages: acidophiles, 39.71%; basophiles, 44.08%; chromophobes, 16.13%. The range of variation within acidophiles was 31.80-48.22%; basophiles, 21.49-52.30%; chromophobes, 7.52-36.80%. Females in breeding condition showed the following average percentages: acidophiles, 32.36%; basophiles, 48.36%;

chromophobes, 19.28%. The range of variation within acidophiles, 27.35–37.48%; basophiles, 46.69–50.40%; chromophobes, 12.12–25.16%.

2. *Juveniles*. The general tendencies exhibited by young opossums (1–150 days) as to cell proportions are these: (1) decrease in chromophobes (undifferentiated cells) with increase in age; (2) increase in chromophiles with increase in age. Basophiles show the greatest increase up to day 100, at which time they impart to the gland the distinct appearance of long term castration. By 125 days most of these basophiles are depleted in the male; in the female the decrease is significant but much less than in the male. It is concluded, since testosterone propionate will deplete this basophilic accumulation at 30, 46, and 93 days, while gonadotropin is effective only to a slight degree at 79 and 125 days, that the pituitary fully differentiates before the gonads become functional as endocrine glands. Thus, in the absence of sex hormones up to day 100 the pituitary takes on a castration appearance which disappears completely in the male by 125 days, and less completely in the female by 125 days, when the testis and ovary begin to liberate hormones.

E. Effects of gonadectomy during development

Castration changes do not occur in the pituitary of animals whose gonads have not yet begun to function in an endocrine capacity. The striking basophilic depletion which occurs between 100 and 125 days is due to the inception of sex hormone function.

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PLATE 1

EXPLANATION OF FIGURES

Line tracings were made with a Bioscope at 20 X. Heavy stippling represents the anterior lobe; light stippling, posterior lobe, stalk, and diencephalon; cross hatching, pars tuberalis; and heavy line, pars intermedia.

1 Sagittal section of adult opossum pituitary showing: AA, acidophilic region of anterior lobe; AB, basophilic region of anterior lobe; IL, intermediate lobe; IR, infundibular recess; PL, posterior lobe; PT, pars tuberalis; and RL, residual lumen.

2, 3, 4 Transverse sections of adult opossum pituitary at the levels indicated by numbers 2, 3, and 4 in figure 1.

5 Transverse section of pituitary of day-old opossum. Note tubular pars tuberalis and thick sphenoid cartilage.

6, 7 Transverse sections at levels posterior to figure 5 showing large blood sinuses in anterior lobe of day-old animal.

8 Transverse section of 8-day opossum pituitary.

9 Sagittal section of 18-day opossum pituitary. Note thick sphenoid cartilage.

10 Transverse section of 49-day opossum pituitary showing rounded anterior lobe and thin sphenoid bone.

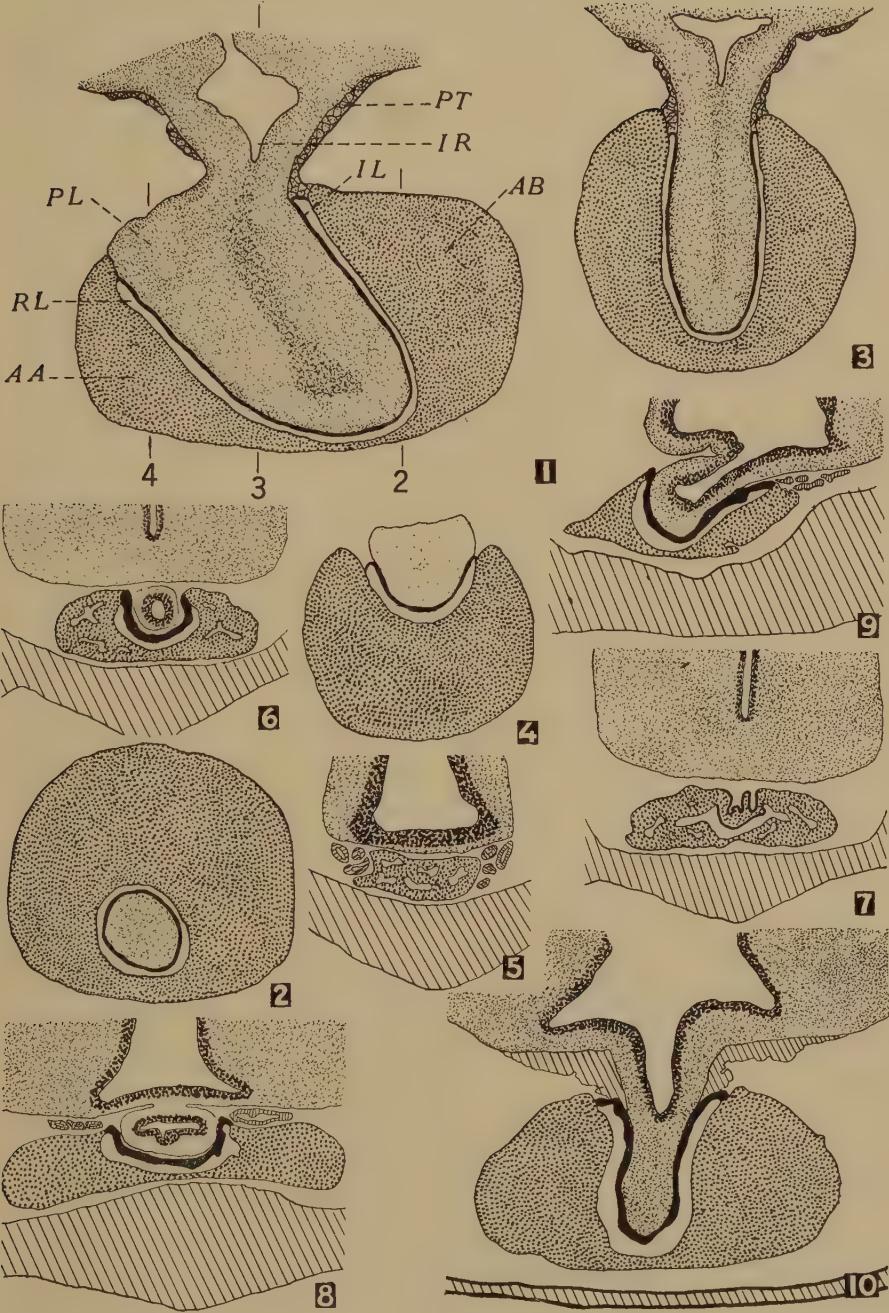


PLATE 2

EXPLANATION OF FIGURES

All photographs $\times 600$.

11 Anterior lobe of normal adult male. Note dark cytoplasmic inclusions in some basophiles.

12 Anterior lobe of normal adult male. Note large blood sinus and vacuolated basophiles.

13 Anterior lobe of adult female gonadectomized 136 days. Note numerous castration cells.

14 Basophilic region of normal anestrus female. Note small size of cells and vacuolated basophiles.

15 Anterior lobe of normal 102-day male. Note huge colloid-filled basophiles and dark hyaline bodies.

16 Anterior lobe normal 125-day male. Section includes part of residual lumen, pars intermedia, and posterior lobe.

